A Randomized Controlled Trial to Measure Spillover Effects of a Combined Water, Sanitation, and Handwashing Intervention in Rural Bangladesh

Jade Benjamin-Chung, Nuhu Amin, Ayse Ercumen, Benjamin F. Arnold, Alan E. Hubbard, Leanne Unicomb, Mahbubur Rahman, Stephen P. Luby, and John M. Colford, Jr.

Correspondence to Dr. Jade Benjamin-Chung, Division of Epidemiology & Biostatistics, School of Public Health, University of California, Berkeley, 101 Haviland Hall, MC #7358, Berkeley, CA 94720-7358, (email: jadebc@berkeley.edu)

Author affiliations: School of Public Health, University of California, Berkeley, Berkeley, CA 94720-7358 (Jade Benjamin-Chung, Ayse Ercumen, Benjamin F. Arnold, Alan E. Hubbard, John M. Colford, Jr.), Infectious Disease Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh (Nuhu Amin, Leanne Unicomb, Mahbubur Rahman), Infectious Diseases & Geographic Medicine, Stanford University, Stanford, CA (Stephen P. Luby).

Funding: Research reported in this publication was supported by the National Institute for Child Health and Human Development of the National Institutes of Health under award number R21HD076216. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflict of interest: The authors declare no conflicts of interest.

Running head: Spillovers of Water, Sanitation, and Handwashing

Abstract

Water, sanitation, and handwashing interventions may confer spillover effects on neighbors of intervention recipients by interrupting pathogen transmission. We measured geographically local spillovers in WASH Benefits, a cluster-randomized trial in rural Bangladesh, by comparing outcomes among neighbors of intervention vs. control participants. WASH Benefits randomly allocated geographically-defined clusters to a compound-level intervention (chlorinated drinking water, upgraded sanitation, and handwashing promotion) or control. From January to August 2015, in 180 clusters, we enrolled 1,799 neighboring children age-matched to trial participants that would have been eligible for WASH Benefits had they been conceived slightly earlier or later. After 28 months of intervention, we quantified fecal indicator bacteria in toy rinse and drinking water samples, measured soil-transmitted helminth infections, and recorded caregiver-reported diarrhea and respiratory illness. Neighbors' characteristics were balanced across arms. The prevalence of detectable *E. coli* in tubewell samples was lower for neighbors of intervention vs. control trial participants (prevalence ratio=0.83; 0.73, 0.95). There was no difference in fecal indicator bacteria prevalence between arms for other environmental samples. Prevalence was similar in neighbors of intervention vs. control participants for soil-transmitted helminth infection, diarrhea, and respiratory illness. A compound-level water, sanitation, and handwashing intervention reduced neighbors' tubewell water contamination but did not impact neighboring children's health.

Keywords: spillover effects, indirect effects, herd effects, water and sanitation, handwashing, soil-transmitted helminths, diarrhea, respiratory illness

Abbreviations: WSH, water sanitation and handwashing; STH, soil-transmitted helminths; PR, prevalence ratio; PD, prevalence difference; CI, confidence interval

Introduction

Improvements in household water quality, handwashing practices, and sanitation (WSH) may reduce the risk of diarrhea (1), soil-transmitted helminth (STH) infection (2) and respiratory illness (3,4). WSH interventions may also reduce illness among neighbors through ―spillover effects” (5) (a.k.a. ―herd effects” (6–8) or ―indirect effects” (9)) resulting from 1) reduced fecal contamination in the environment surrounding intervention recipients, 2) reduced pathogen transmission from intervention recipients to neighbors resulting from recipients' lower disease burden due to intervention, or 3) adoption of promoted health behaviors by neighbors. If WSH interventions reduce illness among both recipients and other individuals, estimates that ignore spillover effects would underestimate the full effect of WSH interventions.

There is a rich literature on herd effects of vaccines (5,7). The literature on spillover effects for other infectious disease interventions, such as school-based deworming (10) and insecticide treated nets (11), is growing (5). While many empirical studies have measured WSH interventions' effects directly on recipients (1–3), few have measured spillover effects of WSH (12–19); these studies used observational designs to measure spillovers, so spillover estimates may be susceptible to bias if there are systematic differences between individuals in close proximity to intervention and individuals serving as controls.

We measured spillover effects of a compound-level combined WSH intervention in an existing, large, rigorously designed trial: the WASH Benefits Bangladesh trial (20). This study measured whether compounds neighboring WASH Benefits intervention recipients had lower environmental contamination and whether their children had a lower prevalence of STH, diarrhea, and respiratory illness compared to children neighboring controls.

Methods

Randomization

We performed a cluster-randomized trial building upon the WASH Benefits Bangladesh study (21,20), which was conducted in Gazipur, Mymensingh, Tangail and Kishoreganj districts of central Bangladesh. These areas were selected because they had low groundwater arsenic and iron (to avoid interference with chlorine water treatment) and no other WSH or nutrition programs. WASH Benefits randomly assigned clusters to: 1) drinking water treatment and safe storage, 2) sanitation, 3) handwashing, 4) combined water + sanitation + handwashing (WSH) 5) nutrition, 6) combined nutrition + WSH, and 7) control (no intervention) (21). WASH Benefits investigators randomized treatment within geographic blocks containing adjacent clusters. An investigator at UC Berkeley (BFA) used a random number generator to randomly assign treatment or control within groups of geographically contiguous clusters. Clusters were separated by at least 1 kilometer to reduce the risk of between-cluster spillovers resulting from reductions in disease transmission or the adoption of interventions in the control group. The study found no evidence of spillovers from the intervention to the control group (20).

We measured geographically local spillovers among neighbors of trial participants in 90 control clusters and 90 combined WSH intervention clusters in WASH Benefits. To measure spillovers, this study focused on the combined WSH intervention because we hypothesized that of all intervention packages in the trial it was most likely to produce spillover effects (Figure 1). We selected control clusters where the main trial planned to collect environmental samples to coordinate data collection efforts and maximize comparability with the main trial. Because interventions included visible hardware, neither the outcome measurement team nor study subjects were masked to intervention assignment.

Participants

In rural Bangladesh, families typically live in clusters of households with a common courtyard. Compounds were eligible for WASH Benefits if a pregnant woman resided there at the time of study enrollment who intended to stay in their village during the follow-up period. The trial followed a birth cohort of ―index” children *(in utero* at enrollment) of enrolled mothers. After 24 months of intervention, there were 6.4 study compounds per cluster on average, and these compounds typically comprised <10% of compounds located within the cluster boundaries. To measure spillovers, we enrolled compounds neighboring WASH Benefits compounds in intervention and control clusters concurrent with primary outcome measurement in the main trial. Neighbors were eligible if a child 0-59 months at the time of spillover study enrollment (just younger or older than the index child cohort) resided there and if they were within 120 steps (2 minutes walking time) of a WASH Benefits compound (Figure 2). We excluded children enrolled in WASH Benefits and children in compounds that shared a courtyard, latrine, or handwashing station with a WASH Benefits compound. Within each cluster, there were typically 6-8 WASH Benefits compounds. For the spillover study, field workers first enrolled the closest eligible neighboring compound adjacent to each WASH Benefits compound; then they enrolled additional compounds, prioritizing those closest to WASH Benefits compounds, until 10 neighboring compounds were enrolled per cluster.

Interventions

Intervention recipients in the combined WSH arm received free chlorine tablets (Aquatabs®; NaDCC, Medentech, Wexford, Ireland), a safe storage vessel to treat and store drinking water, child potties, sani-scoop hoes to remove feces from household, latrine upgrades to a double pit pour-flush latrine for all households in the compound; and handwashing stations including soapy water bottles and detergent soap. Local promoters visited study compounds on average six times per month during the two year follow-up period to encourage intervention uptake. The control arm and spillover study participants did not receive interventions or health promotion.

Procedures

Fieldworkers administered a survey to caregivers of enrolled children at the time of enrollment into the spillover study, concurrent with primary outcome measurements in WASH Benefits (after 28 months of intervention). The survey measured household characteristics, child illness, WSH indicators (e.g., water treatment), and neighbors' knowledge of the WASH Benefits and interactions with WASH Benefits participants and promoters.

Due to political instability in Bangladesh, environmental and biological samples for the spillover study and the WASH Benefits trial were collected 4 months after the survey (after 32 months of intervention) to ensure safe transport and a cold chain. Fourteen children originally enrolled to measure spillovers were not present to provide a stool sample; we enrolled another child in the compound aged 0-5 years to replace these children. All participants in the main trial and spillover study were offered a single dose of albendazole following stool collection regardless of infection status. Albendazole was only offered to main trial and spillover study participants after stool collection. Study children may have also received deworming through the national school-based deworming program. Two slides were prepared from each stool sample and analyzed using Kato-Katz within 30 minutes of slide preparation (22). Laboratory technicians quantified *Ascaris*, hookworm, *Trichuris* ova on each slide. Counts were double-entered into a database by independent technicians. 10% of slides were counted by two technicians, and 5% were counted by a senior parasitologist for quality assurance.

In a subset of 86 control and 80 intervention clusters, fieldworkers collected drinking water samples and recorded water source (tubewell, stored water container or filter, tap). They distributed a non-porous, sterilized toy ball to each enrolled child and collected it 24 hours later. Fieldworkers hung 4.5 feet of sticky fly tape at least four feet from the ground near the latrine and food preparation area in a location away from smoke or stoves and protected from rain; they counted and speciated flies on the tape 24 hours later. Laboratory technicians enumerated *E. coli* and total coliform in water samples and *E. coli* and fecal coliform in toy rinses using membrane filtration. Additional details about field procedures are in Web Appendix 1.

Outcomes

We pre-specified outcome measurement on ClinicalTrials.gov (#NCT02396407). We chose STH prevalence measured approximately 32 months post-intervention as the primary outcome of the spillover study because we believed spillovers were likely to impact STH transmission and because this objectively measured outcome is not subject to information bias. Stool samples with any ova were classified as positive. For each helminth, we quantified eggs per gram by multiplying the sum of egg counts from each of the duplicated slides by 12. We classified infection intensity into categories defined by the World Health Organization based on the number of eggs per gram of stool (moderate/high intensity: ≥5,000 eggs/gram for *Ascaris*, ≥1,000 eggs/gram for hookworm, and ≥2,000 eggs/gram for *Trichuris*) (23).

Secondary outcomes included caregiver-reported 7-day diarrhea and respiratory illness prevalence measured approximately 28 months post-intervention. We defined diarrhea as caregiver's report in the prior 7 days of 3+ loose or watery stools in 24 hours or 1+ stools with blood in 24 hours. We defined respiratory illness as caregiver's report in the prior 7 days of persistent cough or difficulty breathing.

While health outcomes serve as distal spillover effects, we also measured proximal spillover effects on environmental contamination after 32 months of intervention and WSH indicators after 28 months of intervention. Environmental contamination measures included the prevalence of *E. coli* and total coliforms in drinking water, the prevalence of *E. coli* and fecal coliforms in sentinel toy rinses, and the presence and number of synanthropic flies near the latrine and food preparation areas. WSH indicators included self-reported water treatment the day before the interview, storage of drinking water, presence of a latrine with a functional water seal, no visible feces on the latrine slab or floor, presence of a dedicated handwashing location with soap, and no visible dirt on the index child's hands or fingernails.

Sample size

We expected spillover effects to be smaller than effects on intervention recipients, so we powered the study to detect a relative reduction of 2.5-6% in primary outcomes, which was less than the 25% relative reduction expected in the WASH Benefits trial. We assumed prevalence differences for diarrhea (change from 14.2% to 8.2%), *Ascaris* (4.2% to 1.7%) and *Trichuris* (11.2% to 7.2%) and intra-cluster correlation coefficients ranging from 0.023 to 0.153 based on observational studies in rural Bangladesh and India (24). Assuming 80% power and a type I error of 0.05, we calculated the required sample size for each outcome of interest, adjusting for the intra-cluster correlation coefficient. Given these assumptions, the spillover study planned to enroll 2,000 children in 180 clusters (90 per arm).

Statistical analyses

Two investigators (JBC, AE) independently conducted an analysis of primary and secondary outcome datasets masked to treatment assignment following a pre-specified analysis protocol, which describes our analysis in full (25). Here, we provide an overview of our analysis.

Analysis was intention-to-treat. Since WASH Benefits eligibility depended on pregnancy timing, we expected trial participants and adjacent neighbors to be equivalent on average except for their proximity to the WSH intervention, allowing us to make inferences about spillover effects by relying only on the cluster randomization. Our primary analysis estimated unadjusted prevalence ratios and differences for binary outcomes (21) and unadjusted fecal egg count reduction ratios (1-ratio of mean intensity in intervention vs. control arm neighbors) for fecal egg counts. Our secondary analysis adjusted for covariates with bivariate associations with each outcome (likelihood ratio test p-value<0.2) (26). We excluded binary covariates with prevalence <5%. We estimated parameters using targeted maximum likelihood estimation with influence-curve based standard errors accounting for clustering (21). Analysts were masked to intervention assignment until results were replicated.

We assumed children were missing at random and conducted a complete-case analysis. For outcomes with loss to follow-up exceeding 20% of the planned sample, we used targeted maximum likelihood estimation to conduct an inverse probability of censoring-weighted analysis, which re-weights measured outcomes to reconstruct the original study population as if no children had missing outcomes (27).

We assessed effect modification by pre-specified covariates: Euclidian distance to the nearest WASH Benefits compound, number of steps to the nearest WASH Benefits compound, presence of natural physical boundaries (e.g., pond) between spillover compounds and the nearest WASH Benefits compound, and the density of WASH Benefits compounds within a given radius of each spillover compound. All statistical analyses were completed using R version 3.2.3.

Human subjects protection

We received approval from the Institutional Review Boards at the University of California, Berkeley (2011-09-3652), the International Centre for Diarrheal Disease Research, Bangladesh (PR-11063), and Stanford University (25863). Participation of human subjects did not occur until after written informed consent was obtained.

<u>Results</u>

The spillover study screened 6,329 compounds neighboring WASH Benefits compounds for eligibility (Figure 3). Field workers enrolled 900 children in 90 control clusters (―control neighbors") and 899 children in 90 WSH clusters (―intervention neighbors"). Overall, 75% (N=634 control neighbors and N=710 intervention neighbors) of enrollees provided a stool specimen.

Characteristics of intervention neighbors and control neighbors enrolled in the spillover study were balanced by randomization, and neighbors' characteristics were similar to those of WASH Benefits participants' (Table 1). Self-reported deworming was balanced across arms among WASH Benefits participants and children in the spillover study. 815 (91%) intervention neighbors and 483 (54%) control neighbors knew of the WASH Benefits study (Web Table 1-2). Among intervention neighbors, 26% had spoken with WASH Benefits participants and 9% had spoken with WASH Benefits promoters about the study. While intervention adherence was high among WASH Benefits study participants at follow-up, there was no evidence of intervention use among intervention neighbors, control neighbors, and WASH Benefits control compounds at 2-year follow-up (Figure 4).

Median fly counts, *E. coli* and fecal coliform prevalence, and mean $\log_{10}$ concentrations in sentinel toy rinses were similar between intervention and control neighbors (Table 2). The prevalence of *E. coli* detected in drinking water was lower for intervention vs. control neighbors (unadjusted prevalence ratio (PR)=0.88; 95% confidence interval (CI): 0.80, 0.96) (Table 3). This effect was stronger among water samples collected directly from the tubewell (PR=0.83; 95% CI: 0.73, 0.95), and there was no effect among samples from stored drinking water (PR=1.02; 95% CI: 0.95, 1.10). The prevalence of total coliforms was similar between arms in all drinking water samples regardless of water source.

Among neighbors in the control arm, the prevalence of *Ascaris* was 31.4%, hookworm was 3.6%, and *Trichuris* was 3.9% (Table 4). There were no differences in STH prevalence comparing intervention vs. control neighbors: *Ascaris* prevalence difference (PD)=0.00 (95% CI: -0.07, 0.08); hookworm PD=0.01 (95% CI: -0.01, 0.04); *Trichuris* PD=0.02 (95% CI: -0.02, 0.05); and any STH infection PD=0.02 (95% CI: -0.05, 0.09) (Table 4, Figure 5). There were also no reductions in geometric fecal egg counts (Table 5). Prevalence of moderate or heavy infections was <5% for all helminths among both intervention and control neighbors (Web Table 3). 4% of control neighbors (N=634) and 5% of intervention neighbors (N=711) were infected with more than one helminth. The prevalence was also similar among neighbors in intervention vs. control arms for diarrhea (8.0% vs. 7.6%) and respiratory illness (8.6% vs. 9.2%) (Table 4).

Adjusted and inverse probability of censoring weighted analyses produced similar results (Web Table 4). For all outcomes, prevalence ratios and differences comparing neighbors of intervention vs. control were similar across levels of effect modifiers (Web Figures 1-7).

Discussion

We measured spillover effects of a combined WSH intervention on environmental contamination, hygienic behavior, and infectious outcomes in young children. By enrolling neighbors of randomly allocated trial participants, our study design enabled us to estimate geographically local spillover effects while relying on the original trial's randomization for inference. We hypothesized that spillovers would occur through three possible mechanisms: 1) reduced environmental fecal contamination, 2) reduced pathogen transmission from intervention recipients to neighbors resulting from recipients' lower disease burden due to intervention, or 3) behavior change among neighbors. We found evidence of spillovers through the environmental mechanism: neighbors of intervention recipients were less likely to have *E. coli* detected in their tubewell water. However, there was no evidence of reductions in other measures of environmental contamination or of STH infection, diarrhea, or respiratory illness among intervention neighbors compared to control neighbors.

Our environmental assessment measured proximal spillover effects on environmental contamination. We found lower *E. coli* concentration in tubewells of intervention neighbors compared to control neighbors. Though we did not find reductions in total coliforms in tubewell water, this indicator includes bacteria not of fecal origin (28) and is less sensitive to changes in fecal input into the environment than *E. coli*. Improvements in latrine infrastructure may have reduced leakage into the groundwater (29); past studies have found fecal indicator bacteria in groundwater up to 2 m from pit latrines and up to 24.5 m in sandy soil (30). We did not find reductions in environmental contamination as measured by fly density, sentinel toys, and stored water, which capture surface level contamination. Together, these findings suggest possible spillover effects through groundwater but not surface level environmental contamination. Secondary contamination through poor hand hygiene, for example, may have counteracted improvements to source water quality.

Spillover effects may also have occurred if neighbors adopted interventions, but we found no evidence of intervention or behavior adoption. Limited hardware availability and lack of resources to purchase hardware likely inhibited diffusion of interventions to neighbors. Dual pit latrines would have been costly for neighbors to construct themselves, and Aquatabs® and the water storage container delivered by WASH Benefits were not sold locally. Spillover effects may have been more likely if neighbors discussed interventions with WASH Benefits participants or saw them in use; however, only 26% of neighbors reported discussing WASH Benefits with intervention recipients. Finally, the absence of behavior change among neighbors may reflect limited knowledge of or perceived harm of illness or low social desirability of the WASH Benefits interventions (31).

There are several features of this study that limit the generalizability of our findings. First, the intervention was only delivered to approximately 10% of each cluster on average. The baseline survey of WASH Benefits households, which was fairly representative of study clusters as a whole, found that intervention coverage was approximately 30% for the water and handwashing components and 20% for the sanitation component, as measured by indicators in Figure 4. Thus, by two-year follow-up, when we measured spillover effects, overall intervention coverage in study clusters was likely to be under 50%. Studies have found that WSH interventions delivered to entire populations (e.g., introduction of municipal piped water and sewerage) were associated with reduced enteric infection (14–17). It is possible that a higher level of intervention coverage must be reached for WSH interventions to yield spillover effects. This is true for vaccines, many of which confer benefits to non-recipients once immunization coverage reaches a herd immunity threshold (typically over 75%) (7). Some vaccines provide indirect protection to unvaccinated individuals at coverage levels below the herd immunity threshold.

Second, the original WASH Benefits study found that the combined WSH intervention led to small reductions in STH prevalence (PD=-3.5%; 95% CI: -7.5, 0.5) and diarrhea (PD= -1.7%; 95% CI: -2.9, -0.6) (20). The size of spillover effects may be correlated with the size of effects on intervention recipients (32); for example, a large reduction in environmental contamination among intervention recipients would be more likely to translate into large spillover effects for neighbors than a small reduction for intervention recipients. However, in this study, impacts on intervention recipients' health and environmental contamination may have been too modest to reduce transmission to neighbors.

Our study is subject to several limitations. First, we measured diarrhea and respiratory illness through caregiver report. Poor recall may have led to misclassification; however, because neighbors did not receive interventions, any misclassification was likely to be non-differential by study arm, which would have biased results towards the null. Second, double-slide Kato-Katz has low sensitivity in low infection intensity settings such as Bangladesh, where large-scale school-based deworming programs have been offered since 2008 (33). This may have limited our statistical power to detect spillover effects, which we would expect to be smaller than effects on intervention recipients. Finally, we did not define social networks. A small body of evidence suggests that enteric and respiratory pathogens can spread through social networks (34); while few studies have examined this for WSH interventions, spillovers through social networks are theoretically plausible (18).

Conclusion

A compound-level combined WSH intervention reduced contamination of neighbors' tubewell water but did not lead to spillovers for other proximal measures of contamination in the domestic environment or for child health outcomes. For proximal spillover effects to translate to distal spillover effects, improvements in neighbors' health behaviors may have been necessary. Alternatively, spillover effects may be more pronounced in populations with higher disease transmission or higher levels of WSH intervention coverage in the community.

Acknowledgements

The authors would like to thank the study participants and fieldworkers who collected the data for the study as well as Md. Nuruzzaman for his oversight of data collection and Veronica Jimenez and Diana Tran for their contributions to figure preparation. The original WASH Benefits trial was supported in part by Global Development grant OPPGD759 from the Bill & Melinda Gates Foundation to the University of California, Berkeley, CA, USA.

References

1. Prüss-Ustün A, Bartram J, Clasen T, et al. Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: a retrospective analysis of data from 145 countries. *Trop. Med. Int. Health*. 2014;19(8):894–905.

2. Strunz EC, Addiss DG, Stocks ME, et al. Water, Sanitation, Hygiene, and Soil-Transmitted Helminth Infection: A Systematic Review and Meta-Analysis. *PLoS Med*. 2014;11(3):e1001620.

3. Rabie T, Curtis V. Handwashing and risk of respiratory infections: a quantitative systematic review. *Trop. Med. Int. Health*. 2006;11(3):258–267.

4. Aiello AE, Coulborn RM, Perez V, et al. Effect of Hand Hygiene on Infectious Disease Risk in the Community Setting: A Meta-Analysis. *Am. J. Public Health*. 2008;98(8):1372–1381.

5. Benjamin-Chung J, Abedin J, Berger D, et al. Spillover effects on health outcomes in low- and middle-income countries: a systematic review. *Int. J. Epidemiol*. 2017;46(4):1251–1276.

6. John TJ, Samuel R. Herd immunity and herd effect: new insights and definitions. *Eur. J. Epidemiol.* 2000;16(7):601–606.

7. Fine PE. Herd immunity: history, theory, practice. *Epidemiol. Rev.* 1993;15(2):265–302.

8. Fox JP, Elveback L, Scott W, et al. Herd immunity: basic concept and relevance to public health immunization practices. 1971. *Am. J. Epidemiol.* 1995;141(3):187-197; discussion 185–186.

9. Halloran ME, Haber M, Longini IM Jr, et al. Direct and indirect effects in vaccine efficacy and effectiveness. *Am. J. Epidemiol.* 1991;133(4):323–331.

10. Miguel E, Kremer M. Worms: Identifying Impacts on Education and Health in the Presence of Treatment Externalities. *Econometrica*. 2004;72(1):159–217.

11. Hawley WA, Phillips-Howard PA, Kuile FOT, et al. Community-Wide Effects of Permethrin-Treated Bed Nets on Child Mortality and Malaria Morbidity in Western Kenya. *Am. J. Trop. Med. Hyg.* 2003;68(4 suppl):121–127.

12. Aziz KMA, Hoque BA, Hasan KZ, et al. Reduction in diarrhoeal diseases in children in rural Bangladesh by environmental and behavioural modifications. *Trans. R. Soc. Trop. Med. Hyg.* 1990;84(3):433–438.

13. Alderman H, Hentschel J, Sabates R. With the help of one's neighbors: externalities in the production of nutrition in Peru. *Soc. Sci. Med.* 2003;56(10):2019–2031.

14. Macassa G, Ponce de Leon A, Burström B. The impact of water supply and sanitation on area differentials in the decline of diarrhoeal disease mortality among infants in Stockholm 1878—1925. *Scand. J. Soc. Med.* 2006;34(5):526–533.

15. Barreto ML, Genser B, Strina A, et al. Effect of city-wide sanitation programme on reduction in rate of childhood diarrhoea in northeast Brazil: assessment by two cohort studies. *The Lancet*. 2007;370(9599):1622–1628.

16. Huq A, Yunus M, Sohel SS, et al. Simple Sari Cloth Filtration of Water Is Sustainable and Continues To Protect Villagers from Cholera in Matlab, Bangladesh. *mBio*. 2010;1(1):e00034-10.

17. Barreto ML, Genser B, Strina A, et al. Impact of a Citywide Sanitation Program in Northeast Brazil on Intestinal Parasites Infection in Young Children. *Environ. Health Perspect.* 2010;118(11):1637–1642.

18. Kim DA, Hwong AR, Stafford D, et al. Social network targeting to maximise population behaviour change: a cluster randomised controlled trial. *The Lancet*. 2015;386(9989):145–153.

19. Fuller JA, Villamor E, Cevallos W, et al. I get height with a little help from my friends: herd protection from sanitation on child growth in rural Ecuador. *Int. J. Epidemiol.* 2016;dyv368.

20. Luby S, Rahman M, Arnold B, et al. Effects of water quality, sanitation, handwashing and nutritional interventions on diarrhoea and child growth in rural Bangladesh: A cluster randomized trial [available online ahead of print January 29, 2018]. *Lancet Glob. Health*. 2018;DOI:10.1016/S2214-109X(17)30490-4.

21. Arnold BF, Null C, Luby SP, et al. Cluster-randomised controlled trials of individual and combined water, sanitation, hygiene and nutritional interventions in rural Bangladesh and Kenya: the WASH Benefits study design and rationale. *BMJ Open*. 2013;3(8):e003476.

22. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Rev. Inst. Med. Trop. São Paulo*. 1972;14(6):397–400.

23. WHO. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. *World Health Organ. Tech. Rep. Ser*. 2002;912:i–vi, 1-57, back cover.

24. Salvatore A, Patil S. Scaling Up Rural Sanitation: Findings from the Impact Evaluation Baseline Survey in Madhya Pradesh, India. Washington, D.C.: World Bank Water and Sanitation Program (WSP); 2011.

25. Benjamin-Chung J, Arnold B, Ercumen A. Spillover Effects of Water, Sanitation, and Handwashing Interventions on Child Health in Rural Bangladesh: Pre-Specified Analysis Plan. 2016;(https://osf.io/j9xht/). (Accessed February 15, 2018)

26. Pocock SJ, Assmann SE, Enos LE, et al. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems. *Stat. Med.* 2002;21(19):2917–2930.

27. Gruber S, Laan M van der. tmle: An R Package for Targeted Maximum Likelihood Estimation. *UC Berkeley Div. Biostat. Work. Pap. Ser.* [electronic article]. 2011;(http://biostats.bepress.com/ucbbiostat/paper275)

28. Gruber JS, Ercumen A, Jr JMC. Coliform Bacteria as Indicators of Diarrheal Risk in Household Drinking Water: Systematic Review and Meta-Analysis. *PLOS ONE*. 2014;9(9):e107429.

29. Pedley S, Yates M, Schijven J, et al. Pathogens: health relevance, transport and attenuation. In: *Protecting Groundwater for Health: Managing the Quality of Drinking Water Sources*. London: IWA Publishing; 2006:49–80.

30. Crane SR, Moore JA. Bacterial pollution of groundwater: A review. *Water. Air. Soil Pollut.* 1984;22(1):67–83.

31. Dreibelbis R, Winch PJ, Leontsini E, et al. The integrated behavioural model for water, sanitation, and hygiene: a systematic review of behavioural models and a framework for designing and evaluating behaviour change interventions in infrastructure-restricted settings. *BMC Public Health*. 2013;13(1):1015.

32. Benjamin-Chung J, Arnold BF, Berger D, et al. Spillover effects in epidemiology: parameters, study designs and methodological considerations. *Int. J. Epidemiol.* 2018;47(1):332–347.

33. Nikolay B, Brooker SJ, Pullan RL. Sensitivity of diagnostic tests for human soil-transmitted helminth infections: a meta-analysis in the absence of a true gold standard. *Int. J. Parasitol.* 2014;44(11):765–774.

34. Bates SJ, Trostle J, Cevallos WT, et al. Relating Diarrheal Disease to Social Networks and the Geographic Configuration of Communities in Rural Ecuador. *Am. J. Epidemiol.* 2007;166(9):1088–1095.

Figure Legends

Figure 1. Theoretical model for spillover effects of a compound-level combined water, sanitation, and handwashing intervention.
Abbreviation: STH, Soil-transmitted helminth.
Contamination of neighbors' water source and stored water was measured by enumerating fecal indicator bacteria in drinking water samples. Fecal contamination of the neighbors' compound and environment was measured by counting synanthropic flies captured near cooking areas and latrines. Contamination of hands in the neighbors' compound environment was measured by observing caregiver's and children's hand cleanliness. Upwards arrows indicate increases and downwards arrows indicate decreases.

Figure 2. Study design
This figure depicts the study design in two clusters, one assigned to the combined water, sanitation, and handwashing intervention and the other assigned to control. Each cluster was separated by a buffer zone of at least 1 km to minimize the chance of spillovers between clusters. The numbered circles denote the compounds enrolled in the WASH Benefits study. The gray diamonds denote the neighboring compounds enrolled in the spillover study. The WASH Benefits study did not formally define the boundaries of each cluster. In this figure, the darker shaded center of each cluster is the polygon formed by linking the outermost compounds in each cluster, and the lighter shaded section is the periphery around this polygon. We restricted enrollment to the compounds within this periphery to ensure that the 1km buffer zone was maintained in this study.

Figure 3. Participant flowchart

Figure 4. WSH intervention uptake indicators among WASH Benefits and spillover study participants. Improved water quality indicators: A) participant reported treating water yesterday, B) field worker observed stored drinking water in the participant's compound. Improved sanitation indicators: field worker observed C) participant had access to a latrine with a functional water seal, D) no visible feces on the participant's latrine slab or floor. Improved handwashing indicators: field worker observed E) a participant had a handwashing location with soap, F) no visible dirt on study child's hands or fingernails. Circles and diamonds indicate percentage of participants. Vertical lines through each circle and diamond indicate 95% confidence intervals.

Figure 5. Unadjusted prevalence differences for intervention vs. control among intervention recipients and their neighbors
In the main trial, soil-transmitted helminth infection was measured among index children, pre-school age children, and school-aged children; diarrhea was measured among children <36 months in the compound at enrollment; and respiratory illness was measured among index children and all other children under 5 years in the compound two years post-intervention. In the spillover study, all health outcomes were measured in the study child 0-5 years enrolled in the spillover study. Circles and triangles indicate unadjusted prevalence differences. Vertical lines through each circle and triangle indicate 95% confidence intervals.

Table 1. Characteristics of WASH Benefits trial participants and nearby neighbors by intervention group after 28 months of intervention, Bangladesh, 2015

	Neighbors of WASH Benefits participants						WASH Benefits participants					
No. of compounds:	Control (N=900)			Intervention (N=899)			Control (N=1382)			Intervention (N=702)		
Characteristic	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)
Child [a]												
Age (years)			2.3 (1.1)			2.4 (1.1)			1.9 (0.2)			1.9 (0.2)
Female	391	43		439	49		572	50		282	48	
Male	509	57		460	51		562	50		305	52	
Deworming in past 6 months [b]	479	53		507	56		593	64		303	58	
Maternal												
Age			26.4 (5.4)			26.4 (5.3)			25.4 (5.0)			26.1 (5.4)
Years of education			6.1 (3.5)			5.6 (3.4)			5.9 (3.5)			5.9 (3.4)
Paternal												
Years of education			5.2 (4.2)			4.6 (4.2)			4.9 (4.0)			5.1 (4.3)
Works in agriculture	221	25		258	29		296	26		160	27	
Household												
Number of persons per household			5.2 (1.9)			5.2 (1.9)			5.3 (2.1)			5.3 (1.9)
Has electricity	654	73		659	73		833	73		434	74	
Has a cement floor	171	19		121	13		160	14		74	13	
Acres of agricultural land owned			0.11 (0.13)			0.11 (0.17)			0.13 (0.16)			0.13 (0.16)
Average meters to nearest WASH Benefits compound			85 (74)			70 (62)	[c]	[c]	[c]	[c]	[c]	[c]
Average number of steps to nearest WASH Benefits compound			119 (107)			96 (94)	[c]	[c]	[c]	[c]	[c]	[c]
Average number of WASH Benefits compounds within 250 meters			2.7 (1.5)			2.8 (1.5)	[c]	[c]	[c]	[c]	[c]	[c]

[a] Characteristics for spillover children in columns 2-7. Characteristics for WASH Benefits index children in columns 8-13.

[b] Measured after 32 months of intervention, concurrent with stool specimen collection.

[c] Not applicable.

Table 2: Synanthropic fly[a] counts and ratios of fly counts between intervention arms among neighbors after 32 months intervention, Bangladesh, 2015

	Control Neighbors				Intervention Neighbors					
Fly count location	No. Compounds	Median (SD) fly count	No. with any flies	% with any flies	No. Compounds	Median (SD) fly count	No. with any flies	% with any flies	Unadjusted ratio of fly counts	95% CI[b]
Near latrine	717	3 (13)	553	78	713	3 (21)	576	82	1.16	0.81, 1.66
Near cooking area	718	3 (23)	570	79	711	3 (21)	559	79	0.88	0.64, 1.21

[a] Includes *Musca domestica*, bottle flies (*Calliphoridae*), flesh fly (*Sarcophagidae*), lesser house fly (*Fannia canicularis*)

[b] CI: confidence interval. Standard errors account for clustering at the study cluster level.

Table 3: Sentinel toy and drinking water contamination among neighbors after 32 months intervention, Bangladesh, 2015

Measurement	No. Compounds	Mean $\log_{10}$ CFU/ 100 ml[a] (SD)	No. positive samples	% positive samples	No. Compounds	Mean $\log_{10}$ CFU/ 100 ml[a] (SD)	No. positive samples	% positive samples	Unadjusted prevalence ratio	95% CI[b]
Sentinel toys										
E. coli	700	1.5 (1.3)	558	80	697	1.5 (1.3)	581	83	1.05	0.99, 1.11
Fecal coliforms	700	3.4 (1.1)	695	99	697	3.2 (1.2)	691	99	1.00	0.99, 1.01
Drinking water										
E. coli										
All samples [c]	718	0.9 (0.9)	553	77	713	0.7 (1.0)	481	67	0.88	0.80, 0.96
Samples from tubewell	424	0.6 (0.9)	281	66	470	0.4 (0.8)	259	55	0.83	0.73, 0.95
Samples from stored water	258	1.3 (0.8)	238	92	219	1.5 (0.8)	206	94	1.02	0.95, 1.10
Total coliforms										
All samples [c]	718	2.1 (0.5)	710	99	713	2.0 (0.6)	700	98	0.99	0.98, 1.01
Samples from tubewell	424	1.9 (0.6)	416	98	470	1.8 (0.7)	457	97	0.99	0.97, 1.01
Samples from stored water	258	2.3 (0.2)	258	100	219	2.3 (0.1)	219	100	[d]	[d]

[a] For values below the detection limit (1 CFU per 100 mL for water, 2.5 CFU per 100 mL for toy rinses), we imputed 0.5 prior to taking the logarithm.

[b] CI: confidence interval. Standard errors account for clustering at the study cluster level.

[c] Includes n=55 compounds who drew drinking water samples directly from a piped water source, which were not included a separate stratification category due to the low number of observations. 903 (63%) drinking water samples provided by participants were collected from tubewells, 487 (33%) were from stored water, 55 (4%) were from piped water, and 3 (<1%) were from water filters.

[d] Prevalence ratio could not be estimated because all samples contained total coliforms.

Table 4: Prevalence and unadjusted prevalence ratios and differences for diarrhea, respiratory illness, and soil-transmitted helminth infection among children neighboring WASH Benefits compounds after 32 months of WASH Benefits intervention, Bangladesh, 2015

	Control Neighbors		Intervention Neighbors		Unadjusted prevalence ratio[a]	95% CI[b]	Unadjusted prevalence difference[a]	95% CI[b]
Outcome	N	%	N	%				
Diarrhea	898	7.6	897	8.0	1.06	0.76, 1.47	0.00	– 0.02, 0.03
Respiratory illness	898	9.2	897	8.6	0.93	0.63, 1.37	– 0.01	– 0.04, 0.03
Soil-transmitted helminth								
Ascaris lumbricoides	634	31.4	711	31.8	1.01	0.81, 1.27	0.00	– 0.07, 0.08
Hookworm	634	3.6	711	4.8	1.32	0.72, 2.42	0.01	– 0.01, 0.04
Trichuris trichiura	634	3.9	711	5.6	1.43	0.75, 2.72	0.02	– 0.02, 0.05
Any soil-transmitted helminth	634	34.5	711	36.6	1.06	0.86, 1.30	0.02	– 0.05, 0.09

[a] Prevalence ratios and differences compare the prevalence among intervention neighbors to the prevalence in control neighbors.

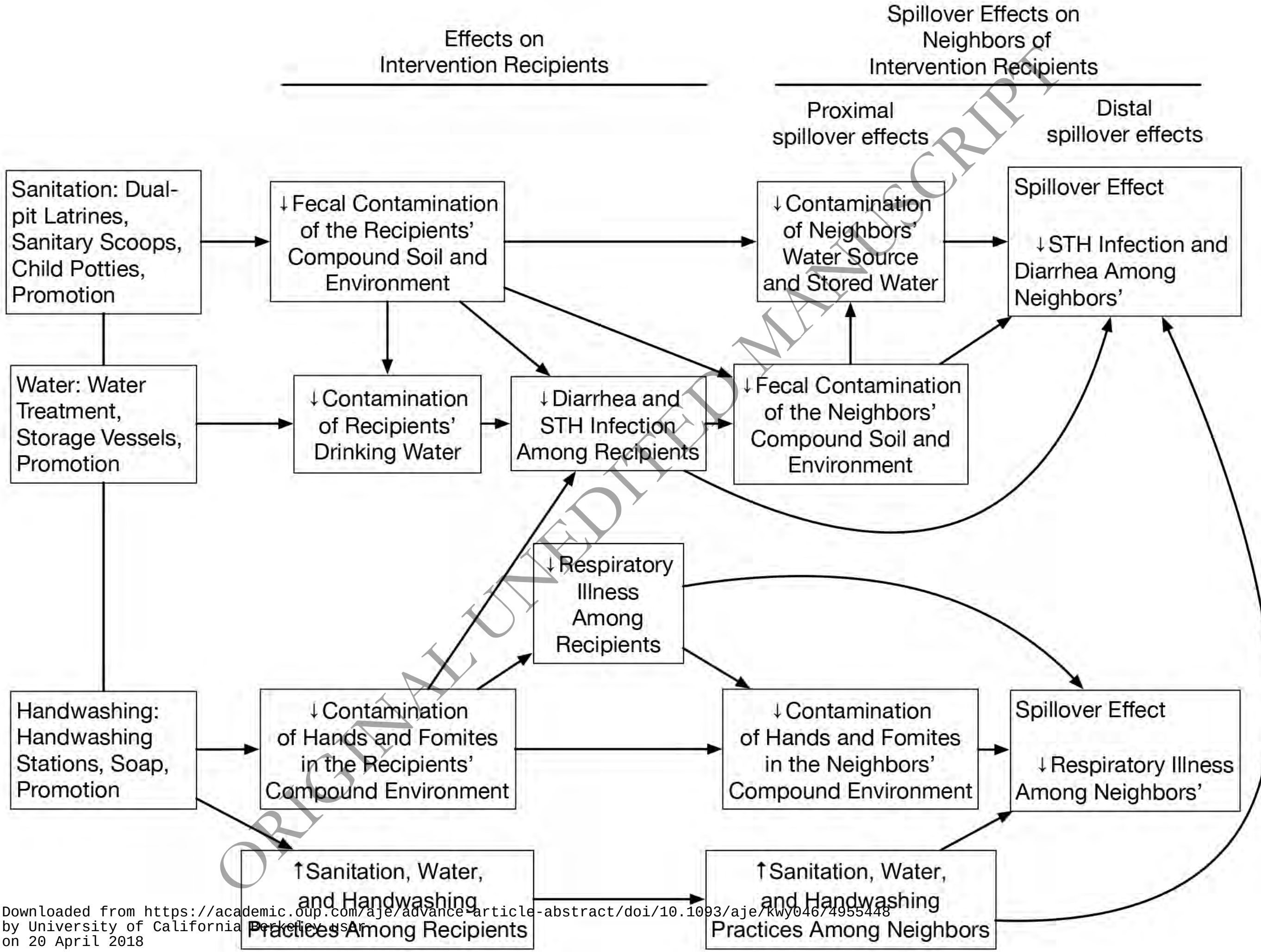
[b] Standard errors account for clustering at the study cluster level.

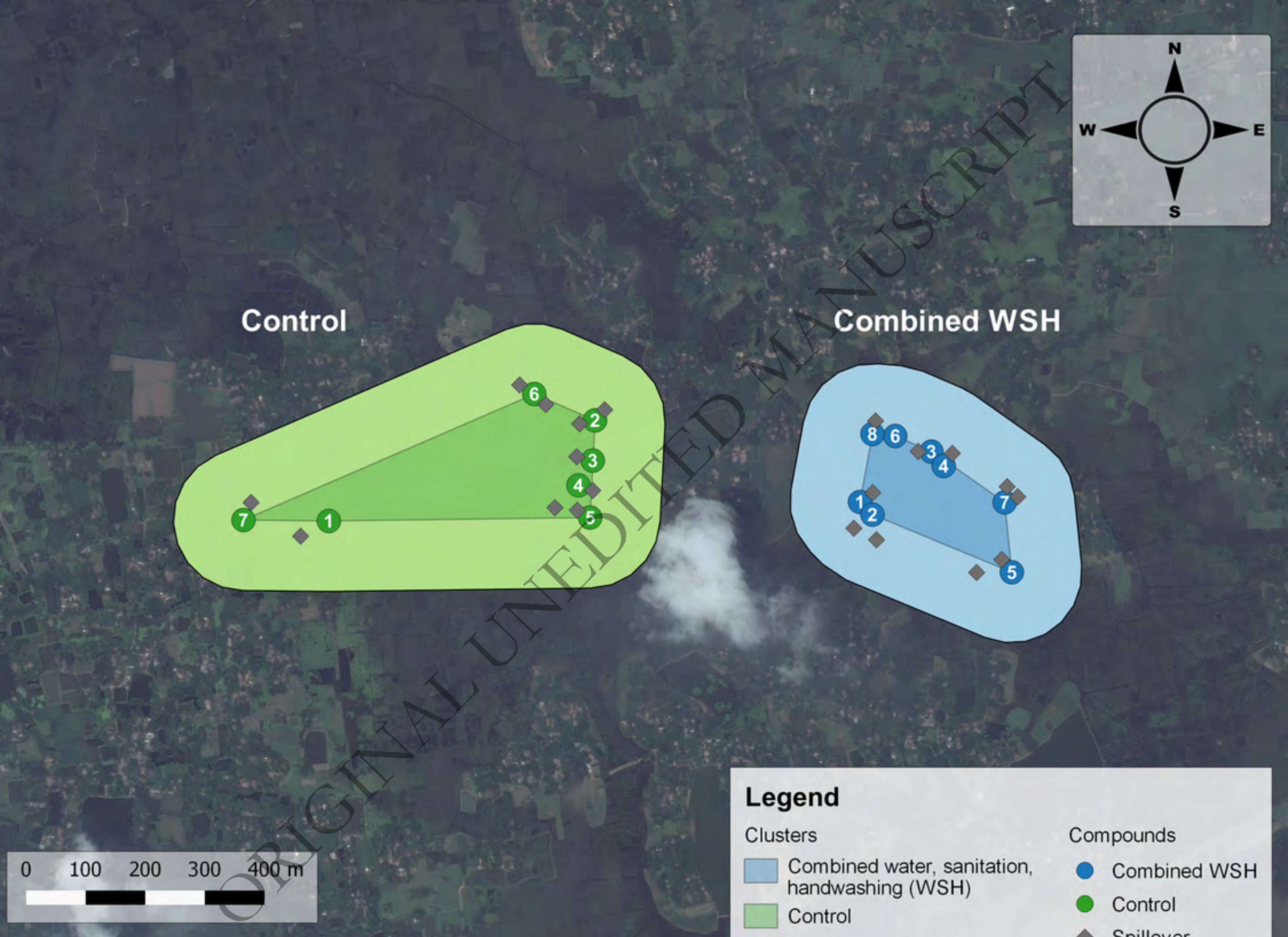
Table 5: Soil-transmitted helminth infection intensity among children neighboring WASH Benefits compounds after 32 months of WASH Benefits intervention, Bangladesh, 2015

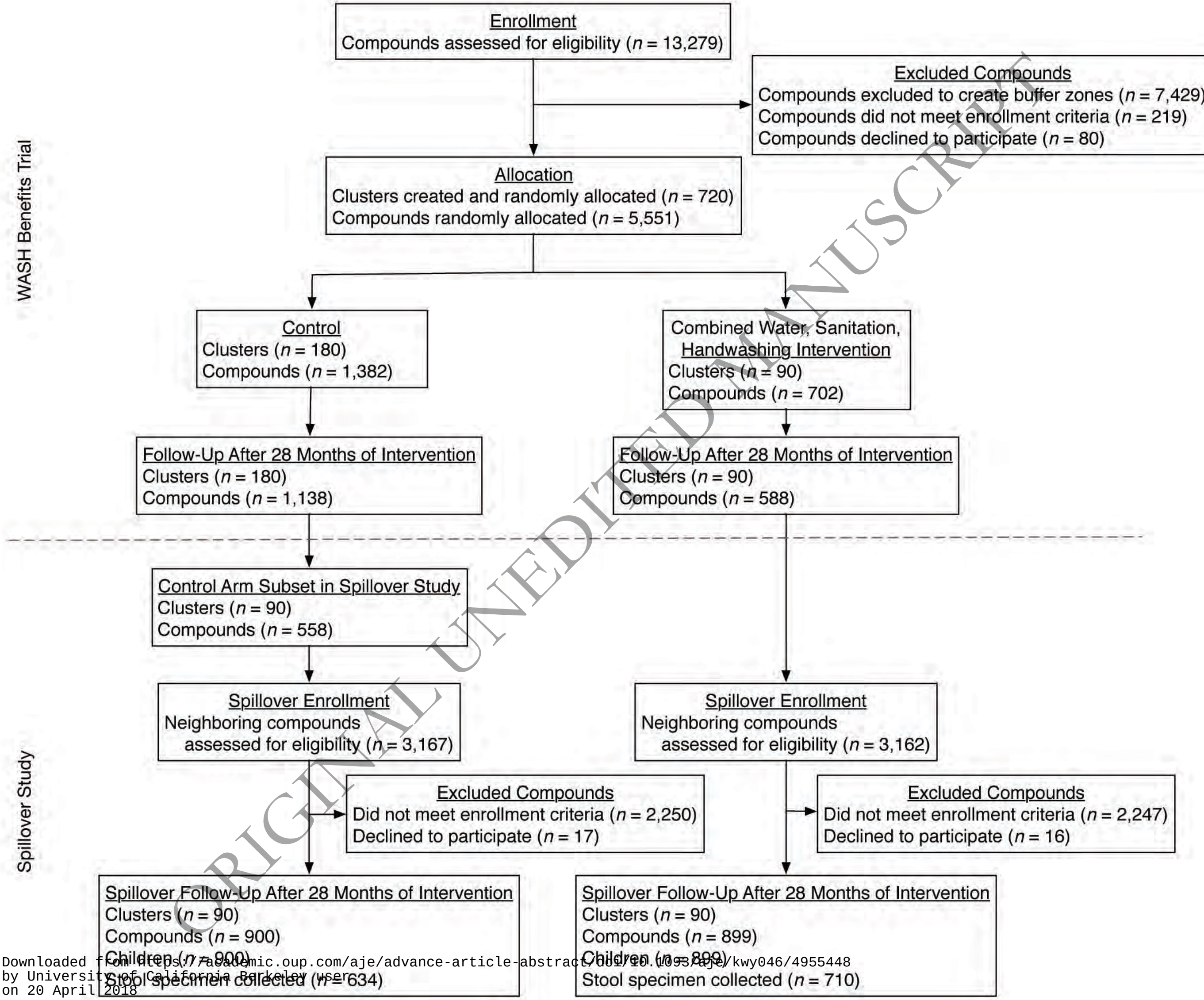
	Control Neighbors		Intervention Neighbors		Fecal egg count reduction ratio[a]	95% CI[b]	Mean fecal egg count difference	95% CI[b]
Soil-transmitted helminth	N	Geometric mean	N	Geometric mean				
Ascaris lumbricoides	634	3.23	711	3.92	0.16	– 0.27, 0.60	0.00	– 0.92, 0.93
Hookworm	634	0.21	711	0.24	0.02	– 0.11, 0.16	– 0.48	– 1.05, 0.10
Trichuris trichiura	634	0.2	711	0.32	0.10	– 0.09, 0.30	2.44	– 2.34, 7.21

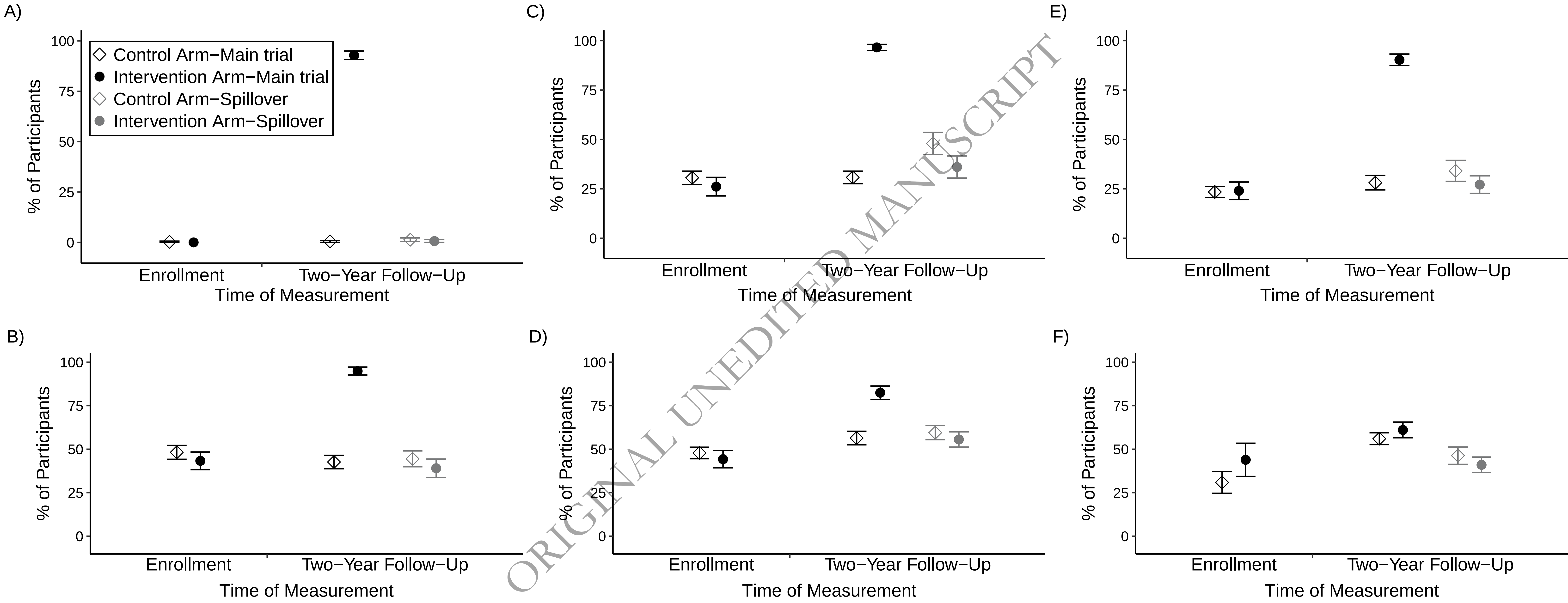
[a] Fecal egg count reduction ratio: (1-RR) x 100%, where the RR is the ratio of mean eggs per gram in the intervention vs. control arm.

[b] Standard errors account for clustering at the study cluster level.

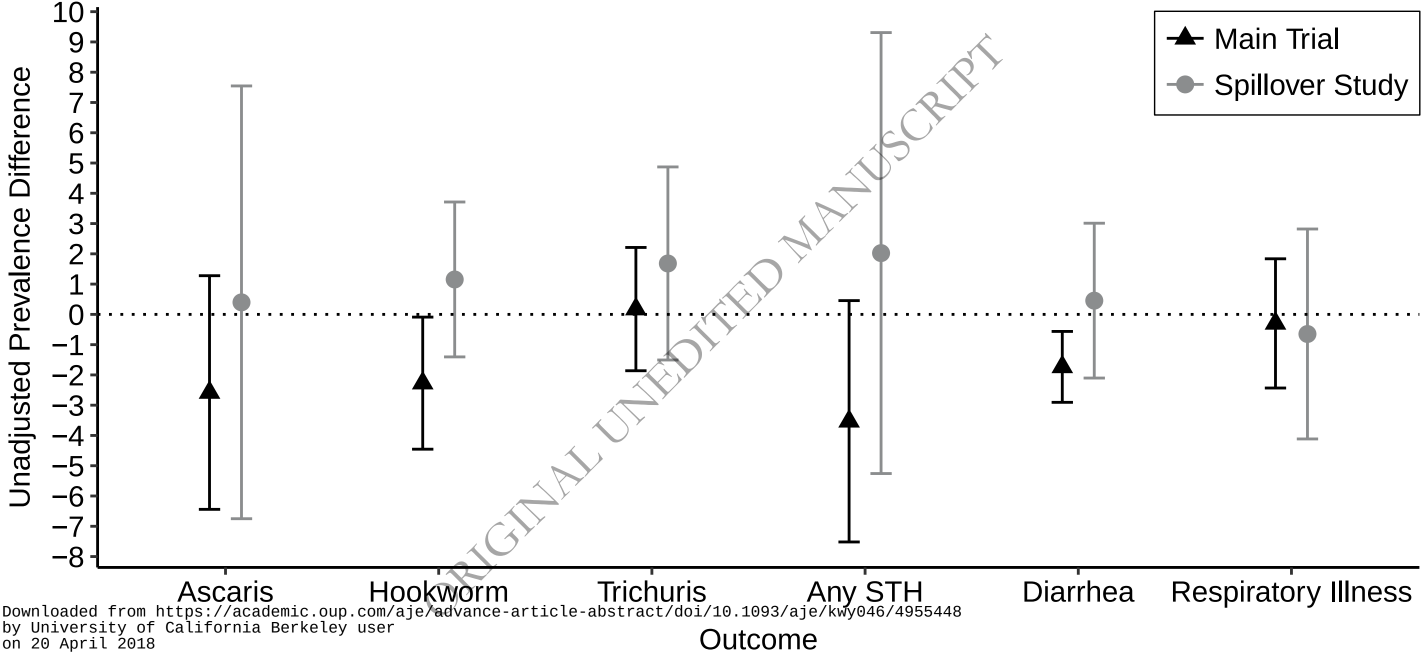

Effects on
Intervention Recipients
Spillover Effects on
Neighbors of
Intervention Recipients
Proximal
spillover effects
Distal
spillover effects
Sanitation: Dual-pit Latrines, Sanitary Scoops, Child Potties, Promotion
↓Fecal Contamination of the Recipients' Compound Soil and Environment
↓Contamination of Neighbors' Water Source and Stored Water
Spillover Effect
↓STH Infection and Diarrhea Among Neighbors'
Water: Water Treatment, Storage Vessels, Promotion
↓Contamination of Recipients' Drinking Water
↓Diarrhea and STH Infection Among Recipients
↓Fecal Contamination of the Neighbors' Compound Soil and Environment
↓Respiratory Illness Among Recipients
Handwashing: Handwashing Stations, Soap, Promotion
↓Contamination of Hands and Fomites in the Recipients' Compound Environment
↓Contamination of Hands and Fomites in the Neighbors' Compound Environment
Spillover Effect
↓Respiratory Illness Among Neighbors'
↑Sanitation, Water, and Handwashing Practices Among Recipients
↑Sanitation, Water, and Handwashing Practices Among Neighbors


Control
Combined WSH
N
W
E
S
Legend
Clusters
Combined water, sanitation, handwashing (WSH)
Control
Compounds
Combined WSH
Control
Spillover
0 100 200 300 400 m


WASH Benefits Trial
Enrollment
Compounds assessed for eligibility (n = 13,279)
Excluded Compounds
Compounds excluded to create buffer zones (n = 7,429)
Compounds did not meet enrollment criteria (n = 219)
Compounds declined to participate (n = 80)
Allocation
Clusters created and randomly allocated (n = 720)
Compounds randomly allocated (n = 5,551)
Control
Clusters (n = 180)
Compounds (n = 1,382)
Combined Water, Sanitation, Handwashing Intervention
Clusters (n = 90)
Compounds (n = 702)
Follow-Up After 28 Months of Intervention
Clusters (n = 180)
Compounds (n = 1,138)
Follow-Up After 28 Months of Intervention
Clusters (n = 90)
Compounds (n = 588)
Spillover Study
Control Arm Subset in Spillover Study
Clusters (n = 90)
Compounds (n = 558)
Spillover Enrollment
Neighboring compounds assessed for eligibility (n = 3,167)
Spillover Enrollment
Neighboring compounds assessed for eligibility (n = 3,162)
Excluded Compounds
Did not meet enrollment criteria (n = 2,250)
Declined to participate (n = 17)
Excluded Compounds
Did not meet enrollment criteria (n = 2,247)
Declined to participate (n = 16)
Spillover Follow-Up After 28 Months of Intervention
Clusters (n = 90)
Compounds (n = 900)
Children (n = 900)
Stool specimen collected (n = 634)
Spillover Follow-Up After 28 Months of Intervention
Clusters (n = 90)
Compounds (n = 899)
Children (n = 899)
Stool specimen collected (n = 710)



A)
B)
C)
D)
E)
F)
Control Arm−Main trial
Intervention Arm−Main trial
Control Arm−Spillover
Intervention Arm−Spillover
% of Participants
100
75
50
25
0
Enrollment
Two−Year Follow−Up
Time of Measurement


Main Trial
Spillover Study
Unadjusted Prevalence Difference
10
9
8
7
6
5
4
3
2
1
0
−1
−2
−3
−4
−5
−6
−7
−8
Ascaris
Hookworm
Trichuris
Any STH
Diarrhea
Respiratory Illness
Outcome